Using Structure-Based Methods For Hit Finding In The Real And Virtual Worlds

Wendy Savory , Jana Wolf, Katie Day, Stuart Firth-Clark, Lydia Lee, Philip Fallon, Martin Bachmann, Kerry Jenkins, Jim Reid, Stefanie Reich, Katie Chapman, Natalie Winfield, Trevor Perrior

Domainex Ltd, Chesterford Research Park, Little Chesterford, Saffron Walden, CB10 1XL

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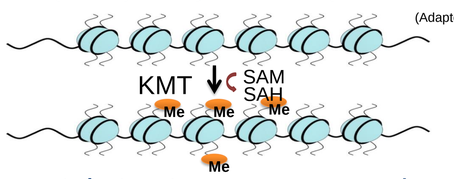
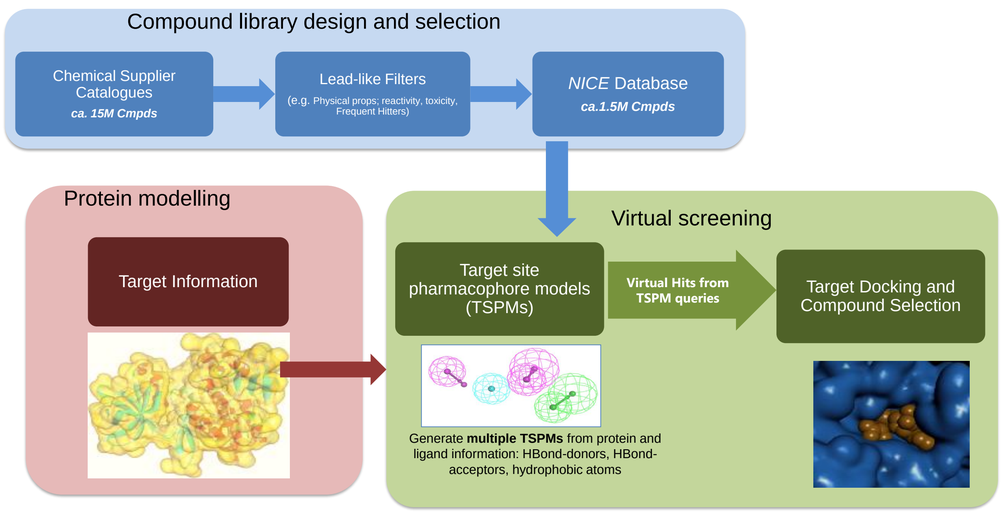
Introduction

- Domainex performs fully-integrated structure-based drug design programs. In this poster, we demonstrate how we use structural information to find small-molecule ligands by both virtual screening (*LeadBuilder*) and fragment screening (*FragmentBuilder*), illustrated with a case study on the lysine methyltransferase (KMT) enzyme, G9 (also known as EHMT2) which is difficult to assay using other biophysical techniques
- G9a is involved in epigenetic gene regulation by covalent modification of histones
- G9a catalyses the transfer of methyl groups from S-adenosyl methionine (SAM) to lysine residues on histone proteins
- Literature supports the role of G9a in mechanisms of carcinogenesis, making it an attractive oncology target[1-4]
- Domainex has solved the key technical drug discovery challenges associated with KMTs, including generating a number of proprietary crystal structures, assays and a novel screening library of small molecule inhibitors

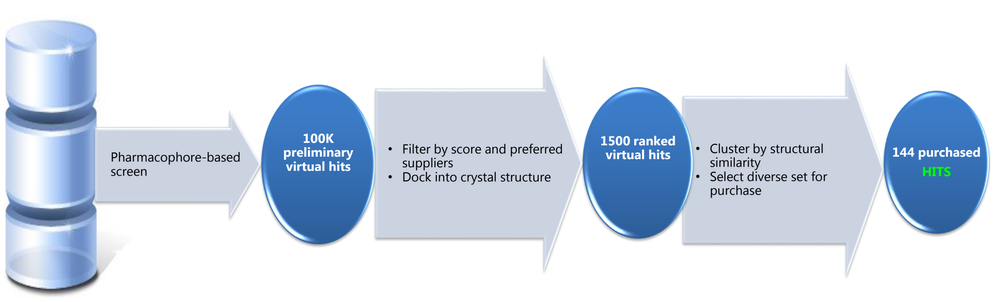
(Adapted & modified from [5])



LeadBuilder – Our Virtual Screen



- Firstly a proprietary crystal structure of G9a bound to a peptide was used to set up *LeadBuilder* to identify small molecule starting points for drug discovery programs
- *LeadBuilder* has two key elements: our in-house curated database of commercially available lead-like compounds and a proprietary two-stage virtual screening protocol based on searches against TSPM, followed by docking into the protein target site
- Typically 500-1500 virtual hits are acquired and tested in an appropriate biochemical assay



- *LeadBuilder* virtual screening identified 144 hits, eight of these tested positive in a biochemical screen. This is equivalent to a hit rate of 5.6%
- One of these hits was optimised and resulted in the discovery of compound B, which has an IC_{50} of 2 nM against G9a
- Crystal structures of G9a in complex with compound B and follow-on compound C are shown (Fig A)

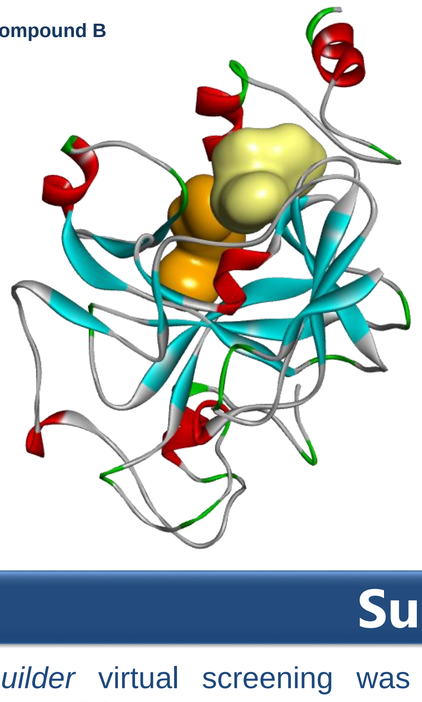
Hit	
G9a	11 µM
IC_{50}	272
MW	0.34
L.E.	

⇨

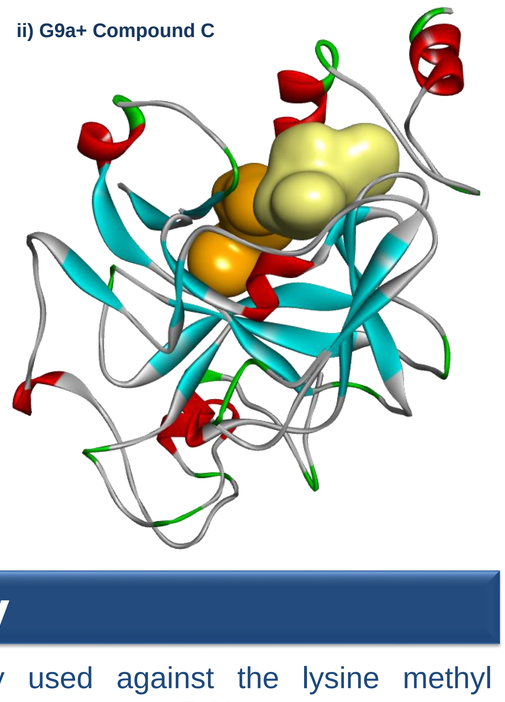
Compound B	
G9a	2 nM
IC_{50}	375
MW	0.43
L.E.	1
HBA	3
HBD	2.5
$LogD_{7.4}$	56
MLM	mins
$t_{1/2}$	

A) In-house X-ray Crystal Structures of G9a + inhibitors arising from elaboration of *LeadBuilder* Hits (Orange –inhibitor, Yellow –SAM)

i) G9a+ Compound B



ii) G9a+ Compound C

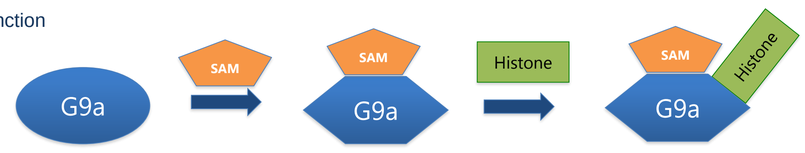


FragmentBuilder – Our Fragment Screen

Advantages of MST over alternative biophysical techniques

	MST	NMR	SPR	DSF	MS	ITC
Protein requirements	V low	Generally high	Low	High	Low	V high
Set-up times	Fast	Medium	Medium-Slow	Fast	Medium	Medium-Slow
Capacity (samples/hr)	Very high (400/hr)	Medium	High	High	Medium	V low
Dynamic range	pM to mM	µM to mM	pM to mM	nM to mM (not quantitative)	pM to mM (not quantitative)	nM to mM
Flexibility	Good – wide buffer choice, works well with tertiary systems	Can use a range of detection methods (waterLOGSY, STD, ^{19}F)	Limited. Protein has to be immobilised, choice of buffers limited.	Limited. Frequently only works with binary systems.	Limited to binary 'binder vs non-binder'. Favours enthalpic over entropic binding.	Limited.
Kinetics (kon, koff)	No	No	Yes	No	No	No

Schematic of G9a function



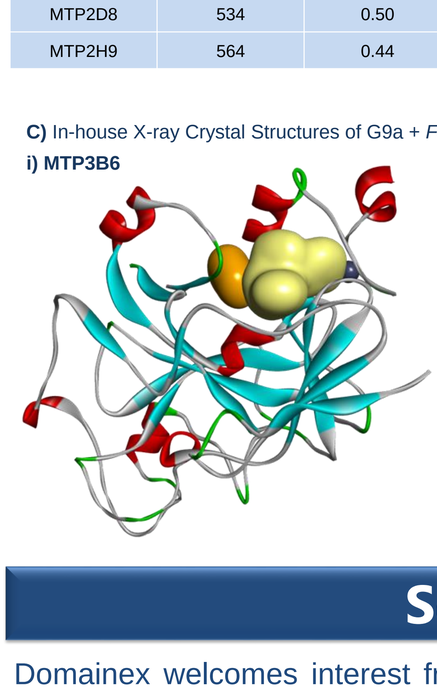
- Microscale Thermophoresis is a powerful biophysical method to quantify biomolecular interactions
- We screened 320 fragments at 1 mM against a G9a-SAM complex using MST
- 17 hits were identified, i.e. a 5.3% hit rate using MST, as compared to 0.3% hit rate when screening the same fragments by DSF or AlphaScreen
- K_d values for 7 hits were also determined without the co-factor, SAM. The K_ds for two fragments were essentially unchanged whereas five fragments showed significant reduction in binding, highlighting the importance of being able to study a ternary system
- Orthogonal confirmation of hit binding to G9a was demonstrated by Saturation Transfer Difference (STD) NMR spectroscopy and in-house X-ray crystallography
- Three fragments were crystallised in the presence of co-factor SAM with a resolution of 1.5- 2.0Å (Fig C) which revealed different binding modes for each fragment
- This has led to several options for FBDD to provide alternative inhibitor chemotypes which are currently under investigation by in-house crystallography

B) G9a Fragment Builder Screening summary

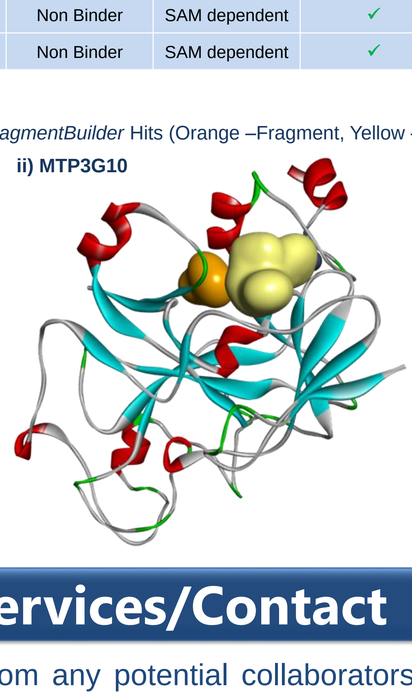
	K_d + SAM	LE (+SAM)	K_d - SAM	Comment	STD-NMR	Crystal trials	X-ray Structure
Frag ID	**K_d [µM]**		**K_d [µM]**	**MOA**	**Positive binding**	**X-ray Structure**	**Resolution**
MTP4E1	117	0.41	94	SAM independent	✓	X	
MTP3G1	718	0.36	518	SAM independent	X		
MTP3B6	17	0.65	109	SAM dependent	✓	✓	1.5 Å
MTP2C3	56	0.41	>1 mM	SAM dependent	X		
MTP3G10	195	0.56	>1 mM	SAM dependent	✓	✓	1.8 Å
MTP2D8	534	0.50	Non Binder	SAM dependent	✓	✓	2.0 Å
MTP2H9	564	0.44	Non Binder	SAM dependent	✓	X	

C) In-house X-ray Crystal Structures of G9a + *FragmentBuilder* Hits (Orange –Fragment, Yellow –SAM)

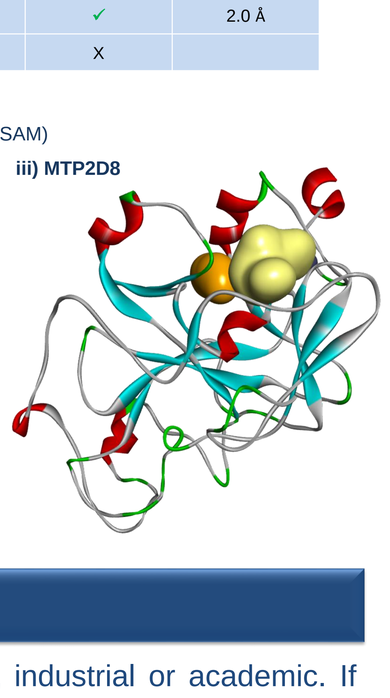
i) MTP3B6



ii) MTP3G10



iii) MTP2D8



Summary

- *LeadBuilder* virtual screening was successfully used against the lysine methyl transferase G9a, to identify small molecule hits with a hit rate of 5.6%
- Microscale Thermophoresis (MST) was successfully used to screen our fragment library, identifying G9a binding fragments with high ligand efficiencies, with a hit rate of 5.3%
- Hit binding was confirmed by biochemical assay or Saturation-Transfer Difference (STD) NMR and X-ray crystallography was used to obtain structural information on the positioning of the compounds/fragments, for hit to lead development
- *LeadBuilder* and *FragmentBuilder* platforms deliver highly-developable hits that enable accelerated hit to lead development

Services/Contact

Domainex welcomes interest from any potential collaborators, industrial or academic. If you would like to learn more about applying our drug-discovery platform to other targets, please contact: **tom.mander@domainex.co.uk**
www.domainex.co.uk

References
[1] Copeland et al., *Nature Reviews*, **2012**, (8), 724-732; [2] Hamamoto et al., *Nature Cell Biology*, **2004**, (6), 731-740; [3] Hamamoto et al., *Cancer Sci.*, **2006**, (97), 113-118; [4] Liu et al. *J. Natl. Cancer. Inst*., **2013**, *doi: 10.1093/jnci/djt30;* [5] Pons et al. *Eur Heart J* 2009 Feb;30(3):266-77